

EXHIBIT Q

Letters

RESEARCH LETTER

Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient

Coronaviruses have been implicated in nosocomial outbreaks¹ with environmental contamination as a route of transmission.²



Supplemental content

Similarly, nosocomial transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reported.³ However, the mode of transmission and extent of environmental contamination are unknown.

Methods | From January 24 to February 4, 2020, 3 patients at the dedicated SARS-CoV-2 outbreak center in Singapore in airborne infection isolation rooms (12 air exchanges per hour) with anterooms and bathrooms had surface environmental samples taken at 26 sites. Personal protective equipment (PPE) samples from study physicians exiting the patient rooms also were collected. Sterile premoistened swabs were used.

Air sampling was done on 2 days using SKC Universal pumps (with 37-mm filter cassettes and 0.3- μ m polytetrafluoroethylene filters for 4 hours at 5 L/min) in the room and anteroom and a Sartorius MD8 microbiological sampler (with gelatin membrane filter for 15 minutes at 6 m³/h) outside the room (eFigure in the Supplement).

Specific real-time reverse transcriptase-polymerase chain reaction (RT-PCR) targeting RNA-dependent RNA polymerase and E genes⁴ was used to detect the presence of SARS-CoV-2 (see detailed methods in the eAppendix in the Supplement). Cycle threshold values, ie, number of cycles required for the fluorescent signal to cross the threshold in RT-PCR, quantified viral load, with lower values indicating higher viral load.

Samples were collected on 5 days over a 2-week period. One patient's room was sampled before routine cleaning and 2 patients' rooms after routine cleaning. Twice-daily cleaning of high-touch areas was done using 5000 ppm of sodium dichloroisocyanurate. The floor was cleaned daily using 1000 ppm of sodium dichloroisocyanurate.

Clinical data (symptoms, day of illness, and RT-PCR results) and timing of cleaning were collected and correlated with sampling results. Percentage positivity was calculated for rooms with positive environmental swabs. Institutional review board approval and written informed consent were obtained as part of a larger multicenter study.

Results | Patient A's room was sampled on days 4 and 10 of illness while the patient was still symptomatic, after routine cleaning. All samples were negative. Patient B was symptomatic on day 8 and asymptomatic on day 11 of illness; samples taken on these 2 days after routine cleaning were negative (Table 1).

Patient C, whose samples were collected before routine cleaning, had positive results, with 13 (87%) of 15 room sites (including air outlet fans) and 3 (60%) of 5 toilet sites (toilet bowl, sink, and door handle) returning positive results (Table 2). Anteroom and corridor samples were negative. Patient C had upper respiratory tract involvement with no pneumonia and had 2 positive stool samples for SARS-CoV-2 on RT-PCR despite not having diarrhea.

Patient C had greater viral shedding, with a cycle threshold value of 25.69 in nasopharyngeal samples compared with 31.31 and 35.33 in patients A and B (Table 1).

Only 1 PPE swab, from the surface of a shoe front, was positive. All other PPE swabs were negative. All air samples were negative.

Discussion | There was extensive environmental contamination by 1 SARS-CoV-2 patient with mild upper respiratory tract involvement. Toilet bowl and sink samples were positive, sug-

Table 1. Sampling Time Points in Relation to Patient Illness and Clinical Cycle Threshold Values

Patient	Days of illness when samples were collected	Presence of symptoms during sampling	Symptoms	Disease severity ^a	Before/after routine cleaning	Cycle threshold value from clinical samples ^b
A	4, 10	Yes, both days	Cough, fever, shortness of breath	Moderate	After	31.31 (day 3); 35.33 (day 9)
B	8, 11	Yes on day 8; asymptomatic on day 11	Cough, fever, sputum production	Moderate	After	32.22 (day 8); not detected (day 11)
C	5	Yes	Cough	Mild	Before	25.69 (day 4)

^a Disease severity was considered moderate if there was lung involvement (opacities on chest radiograph) and severe if patient required supplemental oxygen therapy.

^b Clinical samples were either nasopharyngeal swabs or sputum samples if patient could produce sputum. The most recent result prior to the

environmental sampling was recorded. Cycle threshold refers to the number of cycles required for the fluorescent signal to cross the threshold in reverse transcriptase-polymerase chain reaction; a lower cycle threshold value indicates a higher viral load.

Table 2. Environmental and PPE Sites Sampled and Corresponding RT-PCR Results

Sites ^a	Positive samples (patient C; before routine cleaning) ^b	Cycle threshold value ^c
Environmental sites^d		
Patient's room		
1. Cardiac table, including handle	1/1	35.44
2. Entire length of bed rail	1/1	37.95
3. Control panel on bed	0/1	
4. Call bell attached to bed	0/1	
5. Locker with hand slot	1/1	36.21
6. Chair	1/1	37.07
7. Light switches behind bed	1/1	37.54
8. Stethoscope	1/1	38.24
9. Sink, external rim	1/1	35.54
10. Sink, internal bowl	1/1	36.79
11. Floor	1/1	30.64
12. Glass window in room	1/1	35.79
13. Glass door interior	1/1	35.71
14. PPE storage area over sink	1/1	34.89
15. Air outlet fan	2/3	32.96, 37.94
Toilet area		
16. Door handle	1/1	35.83
17. Toilet bowl, surface	1/1	37.75
18. Hand rail	0/1	
19. Sink, external rim	0/1	
20. Sink, internal bowl	1/1	37.11
Anteroom		
21. Sink, external rim	0/1	
22. Sink, internal bowl	0/1	
23. Floor	0/1	
24. Glass door, room side	0/1	
25. Glass door, corridor side	0/1	
Corridor outside room		
26. Floor	0/1	
Total, No. (%)	17/28 (61)	
Staff PPE sites		
Upper front part of gown	0/2	
Lower front part of gown	0/2	
Front surface of face visor mask	0/2	
Front surface of N95 mask	0/2	
Surface of front of shoes	1/2	38.96

Abbreviations: RT-PCR, reverse transcriptase-polymerase chain reaction; PPE, personal protective equipment.

^a Numbering of environmental sites corresponds to the numbering in the eFigure in the Supplement.

^b Results are shown as number of positive samples/number of total samples. All samples taken from patients A and B after routine cleaning were negative and not included in this table.

^c Cycle threshold refers to the number of cycles required for the fluorescent signal to cross the threshold in RT-PCR; a lower cycle threshold value indicates a higher viral load.

^d One swab was taken from each site except the air outlet fan, from which 3 swabs were taken.

gesting that viral shedding in stool⁵ could be a potential route of transmission. Postcleaning samples were negative, suggesting that current decontamination measures are sufficient.

Air samples were negative despite the extent of environmental contamination. Swabs taken from the air exhaust outlets tested positive, suggesting that small virus-laden droplets may be displaced by airflows and deposited on equipment such as vents. The positive PPE sample was unsurprising because shoe covers are not part of PPE recommendations. The risk of transmission from contaminated footwear is likely low, as evidenced by negative results in the anteroom and clean corridor.

This study has several limitations. First, viral culture was not done to demonstrate viability. Second, due to operational limitations during an outbreak, methodology was inconsistent and sample size was small. Third, the volume of air sampled represents only a small fraction of total volume, and air exchanges in the room would have diluted the presence of SARS-CoV-2 in the air. Further studies are required to confirm these preliminary results.

Significant environmental contamination by patients with SARS-CoV-2 through respiratory droplets and fecal shedding suggests the environment as a potential medium of transmission and supports the need for strict adherence to environmental and hand hygiene.

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